Dynamics studies of tryptophan and single tryptophan containing peptides: simulations and an analytical model

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ABSTRACT

The optimized Rouse-Zimm model is modified leading to a theory for long time random coil polypeptide dynamics [Perico et al., J. Chem. Phys., (1987), 87, 3677, Perico, J. Chem. Phys., (1988), 88, 3996, and Biopolymers, (1989), 28, 1527]. The description necessitates the knowledge of the rotational potential energy for specific amino acid residues and their friction coefficients. Static information, such as the amino acid sequence, the length of the polypeptide chain, and the location of the probe are found to affect the rotational correlation function $P_2(t)$ and the local persistence length markedly. Compared with the fluorescence anisotropy measurements of tryptophan containing polypeptides (of the order of nanoseconds), the theory gives a reasonable prediction for the fluorescence depolarization correlation times of random coil polypeptides, but the calculated rotational correlation function predicts a much faster initial decay and a slower final decay than is observed. Possible theoretical improvements are discussed. Molecular dynamics simulations of tryptophan reorientation are also briefly described.

1. INTRODUCTION

Polypeptides are a class of very interesting molecules to study. Their conformation and structure are strongly dependent on their environment. In general the flexibility of a given polypeptide depends on its amino acid sequence, and this flexibility may have important consequences in, for example, binding at receptor sites.

Among many tools to study polypeptide dynamics, the decay of the fluorescence anisotropy of a specific amino acid residue embedded in the peptide chain provides a convenient experimental approach to study the motion of the polypeptide. Tryptophan, because of its particular photophysical properties, is frequently used as the probe. The tryptophan fluorescence anisotropy decay monitors the rotational relaxation of the indole moiety and has a single exponential with ~35 ps lifetime at room temperature. However, once it is introduced into a peptide chain, this rotation couples with the overall reorientation of the whole molecule, and there is no obvious time scale separation between "internal" and "overall" motions, in contrast to the case for globular proteins. The polypeptides we studied have well-defined double exponential anisotropy decays, ranging from 100 ps to 2 ns, depending on probe locations and environments.

In molecular dynamics simulations, the short time-steps needed to handle the fast motion and the long runs needed to allow evolution of the slower modes make this technique very expensive to apply to polypeptide dynamics. This difficulty can be partly circumvented by treating the surrounding solvent as a heat bath, the so-called Brownian dynamics technique, but the expense of computation time and memory still grows rapidly as the size of the polypeptide system.

(therefore, the number of solvent molecules required) increases and as the experimental time scale is lengthened. Thus, an alternative theoretical treatment is of interest to describe the long time dynamics of large polypeptide (protein) systems.

Theoretically, polymer molecules can be treated as being composed of successive beads and their dynamics in dilute solutions are described traditionally by means of statistical mechanics. Among various models is the Rouse-Zimm approximation\textsuperscript{13,14} which, upon the employment of a projection operator technique that extracts the effective dynamics of the important slow variables, gives a "reduced" description for the center of mass motion of the polymer beads, as in the derivation of the generalized Langevin equation. A further neglect of the memory function leads to the Optimized Rouse-Zimm (ORZ) model\textsuperscript{28}, the solution of which can be obtained by assuming an equilibrium Gaussian conformational distribution. In this picture, the motion of the solvent molecules, although of little interest, is manifested through the inclusion of a solvent viscosity parameter $\eta_0$. The collective motion due to the hydrodynamic interactions of the beads is represented by a draining parameter $\zeta$. In the free draining limit, the perturbation of the flow of solvent by one bead in the chain is taken to have no influence on the flow around any other bead so that there is no hydrodynamic interaction between beads. In the non-free draining case, however, solvent near the center of the macromolecule moves almost in concert with the polymer. Thus, these interior solvent molecules behave as if they were trapped by the high density of polymer segments. As a result, one bead experiences the friction forces exerted by the other beads and has its dynamics strongly affected by their presence. Although it simplifies the calculations greatly, the mathematical approximation of a preaveraged Oseen tensor\textsuperscript{1} in the evaluation of hydrodynamic interactions among the beads introduces some restrictions in addition to those inherent in the polymer model itself.

The ORZ equations require as inputs equilibrium correlation functions for all pairs of amino acid bonds, and an equilibrium average of the hydrodynamic interactions (preaveraged Oseen tensor approximation). For this purpose, we follow Flory\textsuperscript{17} and employ the approximations of neglecting interactions between different residues and of not explicitly averaging over the often multidimensional side group orientations. These approximations may be lifted at the expense of lengthier and more complicated computations. Furthermore, the long time dynamics are properly described provided the correct slow variables have been chosen and the necessary time scale difference exists in the motion of different degrees of freedom of polypeptides.

We have carried out experiments for three 17-residue polypeptides each containing a single tryptophan at positions three, six, and nine and of otherwise identical sequences of leucine and lysine residues. These results, together with our previous anisotropy decay measurements on ACTH, glucagon, and their respective fragments\textsuperscript{3}, enable a test to be made on the validity of our dynamics model.

Brief descriptions of the method for tryptophan MD simulations and of the five simulation systems, along with related results, are given under section 2. Section 3 presents an outline of the theory: polypeptide potential functions and dynamical model. In section 4, a preliminary calculation for ACTH, glucagon, and their respective fragments is discussed. It is believed that the theory reflects the influences of solvent viscosity, probe locations, and chain lengths. A general trend that greater mobility and chain flexibility exist near chain ends is revealed. Experimental data and its comparison with theoretical calculations are included in section 5.

2. TRYPOTHAN DYNAMICS SIMULATION

The amino acid tryptophan has been intensively studied over the last decade. It is interesting not only because of its photophysical properties, but also due to its potential application as a probe in studying biological systems, particularly in dynamical processes. Proper interpretation of experimental data necessitates a thorough understanding of its dynamical behavior as a monomer. It is now well-known that tryptophan has a single exponential fluorescence anisotropy relaxation with a lifetime of about 35 ps although it is complicated by the
overlapped excitation to $^1L_a$ and $^1L_b$ states\textsuperscript{27,34}.

A series of simulations have been done for a single tryptophan in a box of water, two with GROMOS (Groningen molecular simulation system) and three with CHARMM\textsuperscript{21} (chemistry at Harvard molecular mechanics). Except for different algorithms in programming, the main difference lies in the potential functions and interaction parameters employed. For example, although both are 3-site potentials, the SPC/E (originally developed with GROMOS) and TIP3 water models differ in their Lennard-Jones interaction parameters and molecular geometries. Our first and second simulations with GROMOS were devoted to testing the applicability of these two water models.

In the GROMOS force field, hydrogen atoms that are attached to carbon atoms are not explicitly treated, but are incorporated into the latter forming the so-called united atoms. However, a choice exists in CHARMM\textsuperscript{21} between the united atom model and all-hydrogen representations. To assess the influences of these two pictures, we made third and fourth simulations with CHARMM\textsuperscript{21} where tryptophan in its united and full atom representations were calculated respectively.

It is also argued that enough water molecules must be included in order to mimic the dilute aqueous solution of tryptophan. Therefore, in the fifth simulation, about three times as many water molecules were introduced into the system compared with the third and forth one, in hopes of evaluating the effect of system size upon simulations.

An initial configuration for tryptophan was generated either from the x-ray structure, or residue topology and parameter files which are provided by the programs. It was then placed at the center of a cube containing water molecules the number of which varied from simulation to simulation, and therefore, the dimension of box length was adjusted accordingly to reflect the correct water number density at 300\degree K. Water molecules whose oxygen atoms lie within 2 Å (2.6 Å when using CHARMM\textsuperscript{21}) of any heavy atom of tryptophan were deleted entirely. The following table lists the amount of water molecules included in each simulation, together with potential types.

<table>
<thead>
<tr>
<th>Simulation</th>
<th>1\textsuperscript{a})</th>
<th>2\textsuperscript{a})</th>
<th>3\textsuperscript{b})</th>
<th>4\textsuperscript{b})</th>
<th>5\textsuperscript{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td># of water</td>
<td>247</td>
<td>247</td>
<td>112</td>
<td>113</td>
<td>352</td>
</tr>
<tr>
<td>potential</td>
<td>SPC/E</td>
<td>TIP3</td>
<td>TIP3</td>
<td>TIP3</td>
<td>TIP3</td>
</tr>
<tr>
<td>atom type</td>
<td>united</td>
<td>united</td>
<td>united</td>
<td>full</td>
<td>full</td>
</tr>
<tr>
<td>program</td>
<td>GROMOS</td>
<td>GROMOS</td>
<td>CHARMM</td>
<td>CHARMM</td>
<td>CHARMM</td>
</tr>
<tr>
<td>$\langle \tau \rangle_{L_a}$</td>
<td>5ps±1</td>
<td>5ps±1</td>
<td>40ps±8</td>
<td>36ps±7</td>
<td>40ps±6</td>
</tr>
<tr>
<td>$\langle \tau \rangle_{L_b}$</td>
<td>6ps±1</td>
<td>6ps±1</td>
<td>60ps±9</td>
<td>55ps±9</td>
<td>60ps±9</td>
</tr>
<tr>
<td>r(O\textsubscript{a})$_{L_a}$</td>
<td>0.4</td>
<td>0.4</td>
<td>0.36</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>r(O\textsubscript{a})$_{L_b}$</td>
<td>0.4</td>
<td>0.4</td>
<td>0.38</td>
<td>0.38</td>
<td>0.38</td>
</tr>
</tbody>
</table>

\textsuperscript{a}) Errors in $\langle \tau \rangle$ calculated from eqn. (2.4) at $t=\langle \tau \rangle$.

\textsuperscript{b}) Errors in $\langle \tau \rangle$ calculated from eqn. (2.4) at $t=10$ps.

During the course of a simulation, bond stretching was suppressed using the SHAKE algorithm such that in integration a step size of 1 fs with CHARMM\textsuperscript{21} and 2 fs with GROMOS could be used. Periodic boundary conditions were applied to minimize artifacts of boundaries to ensure a converged equilibrium. Before an MD run was initialized, each of the five systems was energy minimized to release bad contacts between atoms or highly strained dihedral angles which might have been introduced when constructing the starting configurations. This was followed by equilibrating the system over a certain amount of elapsed time (ranging from 3 ps to 25 ps, depending on the program used in simulations) to establish a conservation in total energy. During this process, the velocities of the atoms (which represent the kinetic energy of the system) were periodically scaled to maintain a constant temperature. The final molecular dynamics simulation then took the equilibrated structure as its initial point and freely evolved to generate a trajectory.
for each of the atoms in the system. Just as with equilibration, a cut-off radius of 8 Å was provided to calculate both electrostatic and van der Waals energies, and provision was made to update the nonbonded and hydrogen bonded interaction lists periodically. The energies, velocities, and coordinates were recorded every 20 fs during the total 90 ps trajectory in each of the simulations.

Fluorescence anisotropy decay measures the time evolution of the second-order Legendre polynomial of the angle between the absorption dipole moment $\mu_A$ at time zero and the emission dipole moment $\mu_E$ at time $t$

$$r(t) = 2/5 <P_2[\mu_A(0) \cdot \mu_E(t)]> \quad (2.1)$$

It is therefore possible, given the trajectory of the transition dipole moments, to calculate the correlation function of $P_2$ at time $t_m$ using the ergodic assumption that an ensemble average can be replaced by an infinite time average if the system reaches equilibrium. The molecular dynamics analog of this correlation function is:

$$P_2(t_m) = r(t_m)/r(0) = <P_2[\mu_A(0) \cdot \mu_E(t_m)]> = \frac{1}{N \cdot m} \sum_{n=1}^{N-m} P_2[\mu_A(t_n) \cdot \mu_E(t_n+t_m)] \quad (2.2)$$

where $N$ and $m$ are, respectively, the total number of time steps and intermediate time steps in the simulations. The vectors $\mu_A$ and $\mu_E$ at time $t_n$ and $t_n+t_m$, respectively, are calculated from the trajectory.

A particular source of error inherent in numerically evaluating this time correlation is associated with the limitation that an ensemble average cannot be fully realized by a time average over a finite time interval. Nonetheless, some specified accuracy can still be achieved in light of lengths of simulations, rates of relaxations, and different averaging algorithms. Assume the finite time interval is $T$ and define a mean relaxation time $<\tau>$ by

$$<\tau> = 2 \int_0^T P_2^2(t) \, dt \quad (2.3)$$

It is possible to show that the uncertainty associated with the normalized ensemble averaged correlation function $P_2(t)$ is given by

$$\sigma(P_2(t)) = \left( \frac{2<\tau>}{T-\tau} \right)^{-1/2} \quad (2.4)$$

Consequently, a time correlation function can be computed with better confidence for the short-time limit, and this equation will be used when we make error estimates for our simulations. If $P_2(t)$ decays exponentially with the relaxation time $<\tau>$, then $P_2(t) = 1/e$ and this is the method we use to estimate relaxation times in the simulations.

Tryptophan is characterized by two low lying $^1L_a$ and $^1L_b$ excited states. The short time behavior of its fluorescence anisotropy decay, being sensitive to excited state dynamics, is found to be complicated by the possible coupling of the two states upon excitation and emission. As an extreme case, here we calculate the rotational correlation functions for $^1L_a$ and $^1L_b$ independently. No coupling is assumed because it should have no effect upon long time behavior. The orientations of $^1L_a$ and $^1L_b$ transition dipoles have been a subject for much research, and the following picture is a common representation.

![Diagram](image)
A relaxation time of ~6 ps can be deduced from the GROMOS simulation with SPC/E water. This is much faster than the experimental value of 35 ps. Although it is quite unlikely that this discrepancy may be due to inadequately modeled hydrogen bonding, it is possible that inaccuracies in the solvent-solute interaction potential cause the fast motion. Similar simulation with TIP3 water gives an almost identical rotational relaxation rate as far as the initial decays are concerned. Because of the small value of $\tau$, a 23% error exists for $P_2(t)$ at $t=6$ ps. Data points of $P_2(t)$ in figure 1 beyond $t=6$ ps should be considered as statistically under-weighted.

Three simulations with CHARMm give very similar results. A fast initial drop in the anisotropy, which corresponds to inertial motion of tryptophan, is followed by a much slower decay. This can barely be seen in the GROMOS simulations. Unlike simulations with GROMOS where virtually no difference exists between calculations for the $^1L_a$ and $^1L_b$ transition, here motions that lead to $^1L_a$ relaxation tend to have larger inertial drops than for $^1L_b$ relaxation (figures 2 and 3). Decays within the first 10 ps are by no means exponential, but seem to be well-defined. By extrapolating down to the point where $P_2(t)=1/e$, we obtain a decay rate of ~40 ps for the $^1L_a$ transition and a somewhat slower rate, ~60 ps, for the $^1L_b$ transition. This is of course in better agreement with the experimental value of 35 ps. We estimate the errors of $P_2(t)$ at 10 ps to be 20% and 15%, respectively, for $^1L_a$ and $^1L_b$ transitions.

Our simulations using CHARMm with different system size and united or full atom representation give rather similar results, which are also close to experimental measurements. These indicate that the CHARM empirical potential quite faithfully describes solvent-solute interactions. We have not tested CHARM simulations with SPC/E water; therefore, we cannot conclude that the inadequacy of our GROMOS simulations results from the failure of SPC/E in modeling solvent-solute interactions. On the other hand, even with the TIP3 water model, the GROMOS calculation fails to give reasonable agreement with experiments. It is therefore not an unreasonable conjecture that the potential functions and parameters in GROMOS may not be properly rescaled for the united-atom model. Neglect of hydrogen atoms on non-polar carbons will inevitably lead to a smaller size of solute molecule and a decrease in interaction energies. Unless a sufficiently large interaction between solvent and solute is maintained when parameterizing potentials, this could possibly cause an abnormally fast motion for solute molecules.

3. EQUILIBRIUM BOND CORRELATION FUNCTION AND DYNAMICS MODEL

3.1 EQUILIBRIUM PROPERTIES REQUIRED AS INPUT TO DYNAMICAL THEORY

Polypeptides are idealized as a succession of rigid bonds of constant length that are at well-defined angles with respect to each other. Rotations about these bonds generate all possible chain conformations which are consistent with the bonding and steric constraints that may be present in the system. A given conformation of the chain is thus specified by the set of dihedral angles that determine relative bond orientations. With reference to the following figure, the symbol $R_i$ denotes the side chains of the amino acid residues. On a given residue $i$, the rotations referred to earlier are those around the bonds NH($i$)-CH($i$), CH($i$)-CO($i$), CO($i$)-NH($i+1$) and CH($i$)-$R_i$, which are denoted by $\phi_i$, $\psi_i$, $\omega_i$, and $\chi_i$, respectively. As a matter of convention, the all-trans conformation of the peptide is defined as that conformation in which all dihedral angles have the value 180°. Therefore, these rotations vary between plus and minus 180 degrees. Side chains with more than one carbon atom introduce additional dihedral angles $\chi(i,j)$, but these are ignored for the moment.
Geometrical representation of a portion of a polypeptide chain in its all-trans conformation. Successive amino acid residues are indexed i-1, i, and i+1. Virtual bonds connecting the a-carbon atoms of these residues are shown by dashed lines.

Our modified ORZ dynamical model chooses as the basic (slow) variables the virtual bonds \( I_i \) (see figure above) between successive amino acid residues. Consequently a central ingredient of the dynamical theory presented here is the equilibrium bond correlation matrix \( \langle I_i \cdot I_j \rangle \), which describes the average projection of the \( i \)-th virtual bond of the molecule onto the \( i \)-th, and which is a measure of the average chain conformation in solution. The operation denoted by \( \langle \cdot \rangle \) represents the usual Boltzmann average over a canonical distribution of chain conformations.

The actual determination of the equilibrium correlations \( \langle I_i \cdot I_j \rangle \) for a sequence of covalently linked amino acids, is in general prohibitively difficult. However, we may assume that the dihedral angles \( \omega_i \) between successive amino acid residues are in the planar trans conformation because of the partially double-bonded character of the -CO-NH- (peptide) bond. Therefore, the dihedral angles of a given amino acid residue are to zeroth order independent of bond rotations in other residues, provided the chain in solution has a random coil configuration without residue specific interactions such as those promoting local helix formation, etc. The assumption of the independence of rotations on different residues translates into the assumption that the orientation of the \( i \)-th virtual bond \( I_i \) depends only on the angles \( \phi_i \) and \( \psi_i \) of the \( i \)-th residue.

The calculation of \( \langle I_i \cdot I_j \rangle \) may now be determined from the peptide potential energy function by using rotation matrices as discussed by Flory. Briefly, the method proceeds as follows: A reference peptide configuration is taken as the all-trans conformation. A right-handed Cartesian coordinate system is attached to each virtual bond vector \( I_i \) according to some suitable convention (here chosen to correspond with Flory's). Any vector may now be written in terms of the coordinate system of any other vector by a series of rotations that brings the two coordinate systems into coincidence. The operation of rotation is described by a matrix \( \mathbf{R} \) whose general form is given by

\[
\mathbf{R}(\alpha, \beta) = \begin{pmatrix}
\cos(\alpha) & \sin(\alpha) & 0 \\
-\sin(\alpha) \cos(\beta) & \cos(\alpha) \cos(\beta) & \sin(\beta) \\
\sin(\alpha) \sin(\beta) & -\cos(\alpha) \sin(\beta) & \cos(\beta)
\end{pmatrix}
\]

where \( \alpha \) and \( \beta \) represent the two angles through which a given vector is to be rotated to bring it into collinearity with another. The projection of the \( (i+k) \)-th virtual bond on virtual bond \( I_i \), separated from it by \( k \) residues, may be shown to be given by

\[
\langle I_i \cdot I_i \rangle = \mathbf{I}(i) \cdot \mathbf{I}(i+1) \cdot \mathbf{R}(i) \cdot \mathbf{R}(i+2) \cdots \mathbf{R}(i+k-1) \cdot \mathbf{R}(i-k) \cdot \mathbf{I}(i+k)
\]

where the \( \mathbf{I}(i) \)'s are products of three rotation matrices \( \mathbf{R}(i) \) that take bond \( I(i+1) \) successively from its own coordinate system into the coordinate system first of the preceding C-C bond, then into that of the N-C bond preceding the C-C bond, and finally into that of the virtual bond \( I_i \) preceding the N-C bond.

The separability approximation invoked earlier implies that \( \mathbf{I}(i) \) is a function only of the angles \( \phi_i \) and \( \psi_i \). Specifically, because three actual bond vectors add to produce the virtual peptide bond vector, \( \mathbf{I}(i) \) is given by the product form

\[
\mathbf{I}(i) = \mathbf{R}_{13} \cdot \mathbf{R}_{12} \cdot \mathbf{R}_{11}
\]

where \( \mathbf{R}_{11} = \mathbf{R}[-22.2, -\psi, 180.0] \), \( \mathbf{R}_{12} = \mathbf{R}[70.0, -\phi] \) and \( \mathbf{R}_{13} = \mathbf{R}[13.2, 0.0] \). The angles \( 22.2^\circ \), \( 70.0^\circ \), and \( 13.2^\circ \), determined empirically by x-ray crystallography, are the values of the constant angles in the CO-NH- (peptide) bond mentioned earlier.

The average \( \langle I_i \cdot I_j \rangle \) now becomes

\[
\langle I_i \cdot I_j \rangle = l^2 e^T \langle \mathbf{I}(i) \cdot \mathbf{I}(i+1) \cdots \mathbf{I}(j-1) \rangle e
\]

where \( l \) is the magnitude of the virtual bond length (about 3.8 Å), \( e \) is the unit vector \((1,0,0)\),
and the superscript T designates a transposed matrix. The average \( < \cdot > \) is the integral from \(-180^\circ\) to \(180^\circ\) over all \( \varphi_i \) and \( \psi_i \) of

\[
\mathbf{I}(i) \cdot \mathbf{I}(i+1) \cdots \mathbf{I}(j-1) \exp[-\{E(i)+E(i+1)+\cdots+E(j-1)\}] / Z
\]

(3.5)

where the argument of the exponential is the sum of the individual residue energies for a given peptide conformation in units of the thermal energy (at temperature \(300^\circ\)K in our calculations), and \( Z \) is the peptide partition function. Thus, excluded volume is neglected in (3.5).

In general \( E(i) \) is a complicated function of all the dihedral angles \( \varphi \) and \( \psi \), but by the separability approximation it may be regarded as a function solely of the dihedral angles of that residue \( E(i) = E(\varphi_i, \psi_i, \chi_i) \). Thus, the average of the product of the \( \mathbf{I}(i)'s \) in (3.4) becomes the product of the average \( \mathbf{I}(i)'s \).

The evaluation of the matrix \( <\mathbf{I}_i \cdot \mathbf{I}_j> \) clearly depends on the choice of the potential that describes chain conformations. In the present calculations, we use the program ECEPP (Empirical Conformational Energy Program for Peptides\(^{18-20}\)) to compute these energies. Here we provide only a few pertinent details.

The energy of a given peptide conformation in ECEPP is first of all calculated only for that part of the total molecular potential energy that depends on dihedral angles. In other words, bond-stretching and bond-bending contributions are ignored. This energy is determined as the sum of electrostatic \( E_s \), non-bonded \( E_{nb} \), hydrogen bonded \( E_{HB} \), and torsional \( E_t \) terms, together with an additional loop-closing term (ignored here) if there exist one or more disulphide bridges in the chain. \( E_s, E_{nb}, \) and \( E_{HB} \) are given by the pairwise sum of interactions between all atoms separated by at least one degree of rotational freedom. They take the following forms:

(a) The electrostatic energy is

\[
E_s = 332.0 \frac{q_i q_j}{DR_{ij}}
\]

(3.6)

where \( q_i \) and \( q_j \) are the charges in electronic units, \( D \) is the dielectric constant (taken to be 2.0), \( R_{ij} \) is the internuclear distance in Angstroms and 332 is a conversion factor to express \( E_s \) in kcal/mol;

(b) the non-bonded energy is

\[
E_{nb} = F A(k,l)/R_{ij}^{1.2} - C(k,l)/R_{ij}^6
\]

(3.7)

where \( A(k,l) \) and \( C(k,l) \) are coefficients specific to each combination of atom types \( k \) and \( l \), and \( F \) is 0.5 or 1.0 according to whether there is either one or more than one rotational degree of freedom between the interacting atom pair;

(c) the hydrogen bond energy is

\[
E_{hb} = a_{HB}/R_{HB}^{12} - b_{HB}/R_{HB}^{10}
\]

(3.8)

where \( a_{HB} \) and \( b_{HB} \) are specific coefficients for the different combinations of donors and acceptors and \( R_{HB} \) is the distance between the hydrogen atom and its associated partner.

Finally, the torsional energy, introduced to correct for discrepancies between predicted and observed rotational barriers, is given by

\[
E_t = u_0 \{1 \pm \cos(\theta)\}
\]

(3.9)

where \( u_0 \) is the difference in kcauls between the experimental barrier height and that calculated from \( E_s, E_{nb}, \) and \( E_{Hb} \), \( \theta \) is the dihedral angle, and \( n \) is the symmetry number of the barrier.

As stated earlier, the average \( <\mathbf{I}_i \cdot \mathbf{I}_j> \) may be written as the product of the average \( \mathbf{I}(i)'s \). The average \( <\mathbf{I}(i)> \) itself is approximated as the Boltzmann sum of \( \mathbf{I}(i) \) over equal 30 degree increments in the angles \( \varphi_i \) and \( \psi_i \), i.e.,

\[
<\mathbf{I}(i)> = \frac{\sum_{\varphi_i,\psi_i} \mathbf{I}(\varphi_i,\psi_i) \exp[-E(\varphi_i,\psi_i)]}{\sum_{\varphi_i,\psi_i} \exp[-E(\varphi_i,\psi_i)]}
\]

(3.10)

The averaging in (3.10) is significantly more accurate than is the use of rotational isomeric type models for the peptide units\(^{17}\).

The present calculations treat all the side chain atoms explicitly, using the ECEPP
potentials to compute interaction energies. However, for the purposes of averaging over \( \psi \) and \( \psi \) (a computationally expensive procedure) the side chain atoms (except those of glycine and proline) are kept fixed at conformations which yield a characteristic ratio \( C \) of the order of 9.0, the value generally believed to characterize several of this class of molecules\(^{21,22}\). For glycine, this expedient is not necessary, as there is no side chain, while for proline, we simply use the average \( \langle I \rangle \) matrix calculated by Flory. While these approximations for the side chains may appear severe, there is no difficulty, in principle, given greater computer time, in averaging over side group orientations \( \chi \). The present analysis is to be understood as representing a first attempt at going beyond the analytically tractable but somewhat artificial models of earlier calculations\(^{23-26}\).

### 3.2 Dynamical Model

The linear polypeptide chain is modelled as a sequence of \( n - 1 \) virtual bonds joining \( n \) beads (the residues) at the spatial positions \( R_i \). Each bead has a friction coefficient \( \zeta_i \). The chain connectivity constraints and torsional potentials are represented by the potential of mean force \( V(\{R_i\}) \) described below. The chain is also subjected to forces that drive its Brownian motion. The friction coefficient \( \zeta_i \) varies with the residue. Experimental values of \( \zeta_i \) are not available for all of the 20 residues. Here, we use available experimental values\(^{40}\) and the friction coefficients are written as \( \zeta_i = 6\pi n \eta_i \), with \( n \) the solvent viscosity and \( \eta \) the hydrodynamic radius. The values of \( \eta \) are summarized in Table 1. Some computations below use the simplification of choosing a single average \( \zeta \).

The bond vector \( I_i \) may be written in terms of the residue positions as
\[
I_i = R_i - R_{i-1}, \quad i = 1, ..., n-1
\]
We associate with each \( I_i \) a time correlation function \( P_2^i(t) \) which can be measured in fluorescence anisotropy experiments. This time correlation function is related to the second Legendre polynomial of the cosine of the angle \( \theta_i(t) \), describing the rotation of the bond vector \( I_i \),
\[
P_2^i(t) = \frac{r^i(t)}{r^i_0} = \langle \frac{3}{2} \cos^2 \theta_i(t) \rangle - \frac{1}{2}
\]
In addition, we define the fundamental time correlation function of the vector \( I_i \) as
\[
M_1^i(t) = \langle I_i(t) \cdot I_i(0) \rangle < I_i^2 >
\]
To calculate these time correlation functions it is necessary to introduce dynamical equations for the bead positions. We seek to use a reduced description which is valid for these longer time scales. Following Bixon and Zwanzig\(^{28}\), Freed\(^{15,29}\) and Perico\(^{23-26}\) and coworkers, such a reduced description in the context of polymers is available by exploiting projection operator methods. The latter methods require the identification of a set of "slow variables" whose dynamics provide the time correlation function on the time scale of interest. Here we take the slow variables to be the virtual bond vectors, but more detailed and computationally lengthier descriptions are possible in which the slow variables are the individual \( \text{NH}(i)-\text{CH}(i) \), \( \text{CH}(i)-\text{CO}(i) \), \( \text{CO}(i)-\text{NH}(i+1) \), and \( \text{CH}(i)-R_i \) bonds, etc.

The results of the projection operator formalism are represented as a set of coupled Langevin equations for the dynamical evolution of the \( R_i(t) \). Upon neglect of the memory function, the Optimized Rouse-Zimm (ORZ) model emerges in a form that is a generalization of the classic Rouse-Zimm model for the dynamics of flexible polymers in dilute solutions\(^{13,14}\).

The ORZ model produces the Langevin equations\(^{23-25}\)
\[
\frac{\partial R_i(t)}{\partial t} + \sigma \sum_{j=0}^{n-1} (H \Delta R)_{ij} R_j(t) = \nu_i^*(t)
\]
(3.14)
describing the time evolution of the bead coordinates under the combined influence of solvent
induced random forces. These forces are responsible for the Gaussian thermal random velocity functions $v_i^*(t)$, of the full intramolecular potential function $V(R_i)$, and of the hydrodynamic interactions (describing the hydrodynamic transmission of friction forces through the solvent). (The unit time scale $\sigma^{-1}$ is discussed below.) The projection operator formalism provides the matrix $A$ of order $n$ in terms of the inverse $U$ of the normalized equilibrium bond correlation matrix of the preceding section

$$U^{-1}_{ij} = \langle l_i l_j \rangle / l^2$$

as

$$A = M \left( \begin{array}{cc} 0 & 0 \\ 0 & U \end{array} \right) M$$

(3.16)

with the matrix $M$ of order $n$ given as

$$M = \left( \begin{array}{cccc} 1/n & 1/n & \cdots & 1/n \\ -1 & 1 & 0 & 0 \\ 0 & -1 & 1 & 0 \\ \vdots & & & \ddots \end{array} \right)$$

(3.17)

The matrix $H$ is the Oseen hydrodynamic interaction matrix, averaged over the polymer configurations and modified from conventional polymer usage to reflect the different friction coefficients for each of the residues,

$$H_{ij} = \zeta_i^{-1} \{ \delta_{ij} + \zeta \zeta_i \langle 1/R_{ij} \rangle (1 - \delta_{ij}) \}$$

(3.18)

with $\zeta_i$ the reduced residue friction coefficient, given by

$$\zeta_i = \zeta / \zeta = n \zeta / \sum_{j=1}^{n-1} \zeta_j$$

and the reduced friction coefficient defined by

$$\zeta_f = \zeta / 6 \pi \eta_0 l$$

(3.19)

The parameter $\zeta_f$ is treated as an adjustable quantity in the computations to simulate the complicated average draining conditions within the polypeptide coil in solution. This procedure is limited to $\zeta_f < 0.5$ because larger values of $\zeta_f$ generate an average Oseen tensor in (3.18) that is not positive definite (as required physically). The unsatisfactory nature of the model (3.18) arises from the treatment of residues as point friction sources in its derivation. The real hydrodynamic dimensions of the beads may be treated using the Rotne-Prager tensor which may enable computations with $\zeta_f > 0.5$.

Equation (2.14) implies that when the time is scaled by $\sigma$

$$\tau = \sigma t$$

(3.20)

the resulting reduced time correlation functions are independent of $\sigma$. The constant $\sigma$ in (3.14) and (3.20) is

$$\sigma = 3 k_B T / l^2 \zeta = k_B T / 2 \pi \eta_0 l^3 \zeta_f$$

(3.21)

where $\sigma$ and $\sigma_i$ are the basic average and residue rate constants of the model. The rate constant $\sigma$ is simply dependent on the virtual bond length $l$ and the average bead friction coefficient $\zeta$. In a quantitative comparison with experiment, $\sigma$ may be taken as an adjustable parameter in order to compensate for approximations inherent in the ORZ approach and in the estimation of the effect of individual friction coefficients $\zeta_i$.

The entries of the equilibrium bond vector correlation matrix (3.15) are computed by the method presented in section 3.1, while the mean inverse distance matrix $<1/R_{ij}>$ in (3.18) is defined in terms of the full intramolecular potential $V(R_{ij})$ by

$$V(R_{ij})$$


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\[ <1/R_i> = \int d\{R_i\} \left(1/R_i\right) \exp\left[-V(\{R_i\})/k_BT\right] \] (3.22)

with the normalization factor (the partition function)

\[ Z^{-1} = \int d\{R_i\} \exp\left[-V(\{R_i\})/k_BT\right] \] (3.23)

and the full potential energy

\[ V(\{R_i\}) = k_BT \sum_{i=0}^{n-1} E(i) \] (3.24)

It is prohibitively difficult to evaluate the average (3.22) using the transfer matrix formalism of section 3.1. Introducing the Gaussian conformational distribution function, we have

\[ <1/R_i> = (6/\pi)^{1/2} <R_i^2>^{-1/2} \] (3.25)

\[ <R_i^2> = \sum_{p,q=1}^{i} U^{-1}_{pq}, \quad j \geq i \] (3.26)

The approximations (3.6, 3.25, and 3.26) imply that all conformational information enters into the ORZ dynamics through the correlation matrix \( U^{-1} \).

The matrix \( U^{-1} \) contains the information on the static rigidity of the polypeptide. In fact, it may be used to introduce the concept of the local persistence length \( P_n^i \) of virtual bond \( i \), defined as the mean projection of the virtual bond \( i \) on the end-to-end vector distance of the chain

\[ P_n^i/l = \sum_{j=1}^{n-1} <l_i,l_j>/l^2 = \sum_{j=1}^{n-1} U_{ij}^{-1} \] (3.27)

This length describes the average number of bonds statistically aligned with bond \( i \).

Solving equation (3.14) by transformation to normal coordinates \( \xi_a \) enables evaluation of the fundamental time correlation function \( M_1(t) \)3

\[ M_1(t) = \sum_{a=1}^{n} (Q_{a} \cdot Q_{a-1})^2 \mu_a^{-1} \exp(-\sigma\lambda_a t) \] (3.28)

The quantities \( Q \) and \( \{\lambda_a\} \) are, respectively, the matrix of eigenvectors and eigenvalues of the product matrix \( H A \), while \( \mu_a^{-1} \) is proportional to the mean square length of the normal mode \( \xi_a \)

\[ <\xi_a^2> = l^2 \mu_a^{-1} \] (3.29)

Note that \( Q \), \( \{\lambda_a\} \), and \( \{\mu_a\} \) completely characterize the ORZ model, and in the simple Gaussian approximation (3.25) for \( <1/R_i> \), they are functions of the equilibrium bond correlation matrix \( U^{-1} \) and the friction coefficients. Thus, given \( U^{-1} \) calculated by the method of section 3.1, these hydrodynamic quantities are solved exactly.

With the ORZ approximation (3.14) to the generalized diffusion equation, the second order time correlation function is given exactly by23

\[ P_2(t) = 1 - 3\{x^2 - x^3 \ (\pi/2) [1 - (2/\pi) \arctan x] \} \] (3.30)

where

\[ x = \left[1 - (M_1(t))^2\right]^{1/2} / M_1(t) \] (3.31)

Equation (3.30) shows that \( P_2(t) \) in the ORZ approximation is a universal function of the bond vector \( l_i \) time correlation function \( M_1(t) \). It would be of considerable interest to measure \( M_1(t) \) experimentally and thereby test the accuracy of the fundamental ORZ approximation result (3.30). Note that when the segmental relaxation is described by \( P_2(t) \), the dependence on the bond position along the chain and on the polymer sequence enters only via \( M_1(t) \) of equation (3.28) through the quantities \( \lambda_a, \mu_a \) and \( (Q_{a} - Q_{a-1})^2 \).

The static and dynamic properties of each virtual bond (or residue) are described in the ORZ model, respectively, by the bond persistence length \( P_n^i \) and by the time correlation function \( P_2(t) \), or by its correlation time

\[ T_2^i = \tau_2^i \sigma^{-1} \int P_2(t) \ dt \] (3.32)

with \( \tau_2^i \) a dimensionless correlation time, independent of \( \sigma \).
4. BOND CORRELATION TIMES AND BOND PERSISTENCE LENGTHS

The accuracy of the ORZ description for polypeptide dynamics is limited by the quality of the intramolecular potential energy $V(R_i)$ and by the number of degrees of freedom that are included in this reduced description. For a random coil polypeptide the treatment given in section 3 may be sufficiently accurate, while for a more organized polypeptide with secondary structure more analysis may be necessary. In this case, structure, induced by weak forces such as hydrogen bond forces, should be taken into account in the computational scheme.

Chain flexibility of polypeptides affects the dimensionless bond persistence length $P_i/l$ and bond correlation time $T_2^i$. A strong correlation between these equilibrium and dynamical measures of local chain flexibility is observed by plotting $P_i/l$ and $T_2^i$ against bond position $i$ along the chain. Figures 4 and 5 present the dimensionless $T_2^i$ and $P_i/l$ for porcine ACTH (1-39) and glucagon (1-29) as a function of the amino acid sequence (assuming $\zeta=\zeta$ and $\zeta_r=0.25$). These plots differ considerably from corresponding plots reported previously using the same approximations for model homopolypeptides. Here the curves are characterized by multiple maxima and minima in irregular positions, while the homopolypeptides curves appear to be bell-shaped because of increased flexibility near chain ends. The curves for $T_2^i$ and $P_i/l$ are rather similar to each other in both figures, with minima and maxima in both curves located at the same positions in the polypeptide sequence. A significant difference is that the maxima and minima are more pronounced in the dynamical case in figure 4. Three domains clearly emerge in ACTH (see figure 4), separated by two deep minima at virtual bonds 12 (VAL 13) and 24 (ASN 25). The first domain (virtual bonds 1-12) is more mobile showing only three maxima in $T_2^i$, with the highest value at $i=7$ (ARG 8) and a doublet at $i=9$ and 11 (GLY 10, PRO 12). The other two domains are more complicated, having five and six pronounced maxima, respectively. Note that the highest maximum lies in the second domain at $i=20$ (LYS 21). The dynamic spectrum of glucagon is less structured, although two minima at $i=11$ (LYS 12) and $i=23$ (VAL), which are less deep than those in ACTH, suggest the presence of three domains.

To assess the length scale of the interactions, figure 6 displays $T_2^i$ and $P_i/l$ for various fragments of the ACTH peptides: ACTH (1-24), ACTH (1-13), ACTH (1-10), and ACTH (5-10). It appears that within the optimized Rouse-Zimm model with assumptions of independent rotations about virtual polypeptide bonds the mobility of the fragments changes in a regular fashion. The major trend, which parallels the influence of shortening the fragment, is attributable to greater chain flexibility near chain ends. Each fragment resembles its predecessor in that it has a similar pattern in $T_2^i$ and $P_i/l$ within the region of the identical chain composition, although with less distinguishable structure. For instance, the $T_2^i$ in figure 6a for ACTH(1-39) and ACTH(1-24) have almost identical values for residues 1 through 12, while residues 12-24 in the fragment have increased mobility because of the chain end effect. A similar behavior is discernible for the ACTH(1-13) fragment in figure 6a, while the differences in $P_i/l$ in figure 6b are much smaller, but display similar trends. This correlation of stiffness and dynamic structure with sequence may be useful in providing insight into more complicated polypeptides.

As implied in eqn. (3.28), the reduced friction coefficient $\zeta_r$ influences $T_2^i$ in two ways. The first one is due to the overall multiplicative factor of $\zeta_r$ in $\sigma^{-1}$ [equation (3.21)] which therefore scales the relaxation time [equation (3.32)]. The second one emerges from the $H$ matrix [equation (3.18)] and describes the degree of fluid draining through the polymer coil. Figures 7 and 8 illustrate how variation of $\zeta_r$ affects the bond correlation time $T_2^i$ for a peptide (TRP9, see section 5) composed of 17 residues which are assumed to have the same friction coefficient at this stage, i.e., $\zeta_r=\zeta$ for all $i$. Figure 7 considers both effects, while figure 8 only focuses upon the draining effect by taking $\zeta_r$ in (3.21) as equal to 0.5. The trends in the two figures with changing $\zeta_r$ are in opposite directions, with the contribution from the rate constant ($\sigma$) being predominant and being responsible for the gradual increase of the bond correlation time with $\zeta_r$ as shown in figure 7. All the curves in figures 7 and 8, although bell-shaped, have wiggles that represent the influence of the amino acid sequences.
The amino acid residues have significant hydrodynamic volumes (see Table 1). Therefore, high values of $\zeta_r$ are expected that are greater than the empirical value $\sim 0.25$ which is characteristic of "synthetic" polymers in hydrocarbon theta solvents. As Figure 7 shows, an increase in $\zeta_r$ by a factor of two gives an increase in $T_2$ which is less than a factor of two, where the difference appears because of the hydrodynamic interactions. Given the estimates of the amino acid friction coefficients $\zeta_i$ according to Table 1, we may scale $\sigma^{-1}$ in (3.21) accordingly, and confine $\zeta_r$ in $H$ to values $\leq 0.5$ that are necessitated by the approximate $H$ matrix of (3.18). This is actually done when comparisons are made with experimental data.

5. EXPERIMENTS AND CALCULATIONS FOR MODEL POLYPEPTIDES

5.1 EXPERIMENTAL

The three 17-residue peptides used in our experiments contain single tryptophan residues located at specific positions along the chain and have the following amino acid sequences:


The peptides were received as lyophilized powder and were used without further purification. Sample solutions are freshly prepared in 0.01 M phosphate buffer at pH 7.0 and at concentrations of $1 \times 10^{-5}$ to $1 \times 10^{-6}$ M to prevent the formation of any secondary structure. Fluorescence lifetime and anisotropy measurements are performed using the time correlated single photon counting technique previously described. A 291 nm excitation pulse with a FWHM of 8-10 ps is generated using a frequency-doubled, cavity-dumped Rhodamine 6G dye laser, synchronously pumped by an argon-ion laser (Coherent), mode-locked at 514.5 nm. Sample fluorescence is monitored by a micro-channel plate PMT (Hamamatsu R2809U) through a JY H20 monochromator (Instruments, SA) with a 8-nm bandpass centered at 360 nm and a W335 cut-off filter. The instrument function is obtained by scattering the excitation light off of an aqueous suspension of non-dairy creamer and has a FWHM of 55 ps. All the experiments were performed at 25±0.5°C, with temperature control provided by a Neslab RTEA thermostat. Decays are collected up to 10,000 counts at the peak channels. Parallel and perpendicular fluorescence decays are analyzed using the simultaneous fitting method to sums of exponentials.

The magic angle fluorescence lifetime data are listed in Table 2. All the curves fit well ($\chi^2 < 1.2$) to a sum of three exponentials. It is interesting to note that the relaxation rates $\tau^{-1}$ for the three components of the fluorescence decays for each of these three peptides are roughly the same within the accuracy of the experiments. The differences in the relative weights ($a_i$) may arise from different TRP environments and chain conformations.

Typical parallel and perpendicular decay curves for TRP3 are shown in figure 9. The anisotropy decays $r(t)$, obtained by simultaneous fitting of the parallel and perpendicular components of the fluorescence decay, are described reasonably well by a sum of two exponentials with $\chi^2 = 1.20$ for TRP3, TRP6, and TRP9. The results are presented in Table 3. For 291 nm excitation with 55 ps resolution, an $r(0)$ (defined as $r_1(0)+r_2(0)$) of 0.13 is expected (see Fig. 10 of Ref. 35). The TRP9 data give $r(0)=0.12$. Except for TRP9, the TRP6 and particularly the TRP3 data give limiting anisotropies substantially below this value, implying that with the present time resolution rapid components in their anisotropy decays may have been missed. Thus, we may attribute the lack of a significant end effect in TRP3 in the free fits (fifth column of Table 3) at least in part, to the limitations of the experimental data. As pointed out earlier, for short anisotropy times the fitting criterion $\chi^2$ is approximately dependent on the product $r(0)<\tau>$. Thus, the value of $<\tau>$ is not very well determined. Defining an average relaxation time $<\tau>=[r_{1}(0)\tau_{1}+r_{2}(0)\tau_{2}]/r(0)$ for $r(0)=0.12$, we obtain $<\tau>=330$ ps and 200 ps for TRP6 and TRP3 respectively (sixth column of Table 3). A much greater decrease in the anisotropy decay time for TRP6 and TRP3 with respect to TRP9 and therefore an end effect are apparent. Single
exponential fits for peptides TRP9, TRP6, and TRP3 with a fixed r(0) of 0.12 yield <t_r>=880 ps, 440 ps, and 210 ps respectively.

The spectra of the peptides are invariant to the tryptophan locations and there seems no reason to suspect that the L_A and L_B spacing of the tryptophyl residue is changed between the different peptides. Consequently, we conclude that the r(0)-constrained <t_r> values are the most realistic and are the ones that should be used in comparison with the calculated values.

5.2 COMPARISON BETWEEN EXPERIMENT AND THEORY

Initial computations choose the reduced bead friction coefficient ζ_r to be 0.25, in accord with experimental data for polymers in theta solvents, and assume identical residue size (ie., ζ_i=ζ). The parameter σ in (3.11) therefore has the value of 0.04695 ps^{-1} at 300^0K. The calculated average relaxation times are 258 ps, 228 ps, and 72 ps, respectively, for TRP9, TRP6, and TRP3, and are significantly lower than the experimental values regardless of whether the averaged relaxation times or only the longer components of the anisotropy decay are used for comparison. Increasing ζ_r and changing σ accordingly increases the computed relaxation times. However, the optimized Rouse-Zimm approach is limited to ζ_r<0.5 for the same reason as is the original Rouse-Zimm model. Calculations using ζ_r=0.5 yield relaxation times of 374 ps, 346 ps, and 121 ps, respectively, values that are still faster than the experimental data, although approaching a better agreement.

Considering the estimated ζ_i according to Table 1, we find that ζ_r is probably in the order of ζ_r=1 for the polypeptides studied. Thus, until there are significant theoretical advances in the treatment of the H-matrix, we are forced to allow ζ_r to vary separately in σ and in the H-matrix in order to investigate how ζ_r affects the calculated rotational relaxation time. There is a significant draining effect in which correlation times are markedly increased as ζ_r is decreased at constant σ (see figure 8). On the other hand, changes in σ^{-1} produce proportional changes in correlation times if the variation of ζ_r in H is neglected. This is understandable because ζ_r affects the relaxation spectrum in a complicated fashion through the hydrodynamic matrix H and its eigenvalues and eigenvectors, while σ enters the exponential decay rates only as a scale factor.

There exist pairs of ζ_r and σ that are found to yield good fits to the experimental data for TRP9, TRP6, and TRP3 individually. However, some compromise must be made if we seek to find a reasonable simultaneous fit for the three compounds. Assuming ζ_r=0.25 as before, but increasing σ^{-1} by a factor of 3, for example, will triple all the calculated data, namely 774 ps, 684 ps, and 216 ps for TRP9, TRP6, and TRP3 correspondingly, as compared to the r(0)-constrained experimental relaxation times of 826 ps, 333 ps, and 200 ps. Note that the theoretical and experimental ratios <t_r>_{TRP3}/<t_r>_{TRP9} are very close (~0.26), indicating that current theory reproduces the relative mobility of the chain end with respect to the middle quite faithfully. The theoretical prediction for TRP6, however, is considerably slower than the experiment value. We do not yet understand the reason for this discrepancy.

Table 4 lists the results calculated by taking into account the individual friction coefficients, together with the experimental counterparts. Within the range in which ζ_r is allowed to vary as required by preaveraging the H matrix, the theoretical calculation gives relaxation times that vary by only a factor of 2. Once again, TRP6 is predicted to show a relatively slower decay, whereas TRP3 and TRP9 display the correct end effect. A difference appears between calculations using individual friction coefficients and an average for all residues. In the former case, the rate constant σ is generated naturally, and reasonable agreements with experiments can be maintained. However, when assuming ζ_i=ζ for all residues, σ must be decreased by a factor of 2 to 3 to achieve an equivalent agreement with experiments. Figure 10 depicts the relaxation lifetimes for the three peptides as a function of the virtual bond locations for different values of ζ_r. It is found that virtual bond 5 (ie., residue 6) is the most sensitive to the existence of tryptophan. Introducing tryptophan at this location, we observe a decrease in the relaxation rate as large as 240 ps whereas at the position 9 the attainable decrease is less than 120 ps and is practically
zero at position 3. Except for the lowest $\zeta_r$ (0.1 in this case), medium values of $\zeta_r$ (0.25~0.5) tend to give better agreement with the experimental measurements\textsuperscript{37}. However, we believe a more realistic value of $\zeta_r$ would approach 1.

In Figure 11, the time dependence of $P_2(t)$ for the three peptides is plotted at $\zeta_r = 0.1$, 0.25, and 0.5. Also plotted are their normalized experimental relaxation functions. All the calculated $P_2(t)$'s can be described reasonably well by triple exponentials with a very rapid component of about 15 ps and with a relative amplitude at about 45%. Because of the time resolution in the present experiments, such a short decay would not be detected directly, but would lead to a decrease in $r(0) = r_1(0) + r_2(0)$. However, the observation of $r(0) = 0.12$ for TRP9 at 291 nm excitation rules out the possibility that a significant short component is missing experimentally, at least for TRP9. Therefore, it appears likely that the theory is incorrect at short times, and this is consistent with the fact that the time scale separation, assumed in projection operator methods, is not longer valid at short time rotational relaxations of these peptides. The other two exponential components in the computed $P_2(t)$ (on the order of ~350 ps and ~3000 ps, respectively) increase with decreasing $\zeta_r$ for each of the three peptides while their weights have a tendency to decrease. The overall effect, however, causes the average $\langle \tau_r \rangle$ to increase with decreasing $\zeta_r$ since the short component makes a negligible contribution to $\langle \tau_r \rangle$. It is worth noting that the longest component in TRP3 has an extremely small weight (~5%). This, combined with the fact that this component decreases slowly from TRP9 to TRP6 to TRP3 under the same $\zeta_r$, renders TRP3 the smallest $\langle \tau_r \rangle$, consistent with the end effect. Therefore, this theory has a sequence dependence of relaxation manifested through both the weight of each time component and the lifetimes.

The advantage of this model is that it can be used to calculate a wide variety of dynamical properties of polymers given only the knowledge of their specific potential energy functions. Here as a first test we choose to investigate rotational relaxation times. The model incorporates sequence information into the force constant matrix $A$ and into the hydrodynamic interaction matrix $H$. In addition, the $H$ matrix takes into account the individual hydrodynamic volumes of the residues. Therefore, this model relates the conformational and hydrodynamic local details to the global polymer dynamics. Two predecessors of the present theory, the ORZ approximation to the freely-jointed and freely-rotating chain, have no rotational barrier about the backbone of the chain; therefore, no detailed local sequence structure is preserved in these simpler models, and this is, of course, not a realistic picture for the dynamics of polypeptide chains. As presented in Ref. 3, this less complete description can serve to rationalize qualitative aspects of fluorescence anisotropy measurements in terms of chain length, stiffness, and the local probe, but it cannot provide any sequence dependent predictions.

As discussed above, model calculations give rotational relaxation times which are consistently shorter than those obtained from experiments. Thus, the ORZ model represents the chain as too flexible. Interactions between the side groups on neighboring residues may introduce additional stiffness, and extensions of the model in this direction will be of considerable interest. It may, however, turn out that the ORZ model always yields an overestimate of chain flexibility due to the inherent Gaussian equilibrium distribution for the model.

The present ORZ model calculations assume that the transition moment is aligned along the segment vector $\langle l \rangle$. This is obviously approximate, since the excitation of the tryptophyl residue is due to the $\pi-\pi^*$ electronic transition of the indole moiety. Thus, side-chain motion undoubtedly contributes to fluorescence depolarization and must be included in a more detailed treatment. Therefore, another step towards the completion of the hierarchy of increasingly faithful ORZ polymer models is to include the side chain motions. It is believed, however, that considerations of side chain motion may introduce an additional fast relaxation and thereby increase the weight of the short decay component. This would tend to increase the discrepancy between the theoretical predictions and experimental measurements. However, if the motion about the dihedral $\chi_1$ and $\chi_2$ side group bonds is sufficiently constrained, this may increase the local persistence length and override the last effect. The inclusion of side chain motions could therefore lead to a better
theoretical description of the polypeptide dynamics. Work in this direction is currently underway.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

37. Synthetic polymers naively would yield \( \zeta_r \) of (3.9) with widely varying values. Nevertheless, experiments on \( \theta \)-chains are well represented by a universal value of \( \zeta_r=0.25 \), perhaps for deeper physical reasons that remain to be understood. Thus, use of \( \zeta_r=0.25 \) may not be unreasonable. Some insight into the question may be gained from Ref. 15 where the memory terms enter as additional non-Markovian friction forces. A Markovian approximation would introduce an additional friction coefficient matrix whose properties would be interesting to study.

Table 1. Amino acid residue volumes and their hydrodynamic radii a), b)

<table>
<thead>
<tr>
<th>Residue</th>
<th>Volume(Å³)</th>
<th>Radius(Å)</th>
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<tbody>
<tr>
<td>ALA</td>
<td>91.5</td>
<td>2.80</td>
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<tr>
<td>ARG</td>
<td>173.4</td>
<td>3.46</td>
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<tr>
<td>ASN</td>
<td>124.5</td>
<td>3.10</td>
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<tr>
<td>ASP</td>
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<td>GLU</td>
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<td>GLY</td>
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<tr>
<td>GLN</td>
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<td>3.38</td>
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<td>HIS</td>
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<td>3.42</td>
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<tr>
<td>LYS</td>
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<td>MET</td>
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<td>PHE</td>
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<td>TYR</td>
<td>203.6</td>
<td>3.65</td>
</tr>
<tr>
<td>VAL</td>
<td>141.7</td>
<td>3.23</td>
</tr>
</tbody>
</table>

a) The amino acid residue friction coefficients are calculated from \( \zeta_i = 6\pi n_{i0} \rho_i \).
b) Ref. 40.

Table 2. Fluorescence lifetimes (in ps) of 17-residue peptides a), b)

<table>
<thead>
<tr>
<th></th>
<th>( a_1 )</th>
<th>( \tau_1 )</th>
<th>( a_2 )</th>
<th>( \tau_2 )</th>
<th>( a_3 )</th>
<th>( \tau )</th>
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<tr>
<td>TRP3</td>
<td>0.49</td>
<td>3549</td>
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<td>1384</td>
<td>0.10</td>
<td>133</td>
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<tr>
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<td>0.58</td>
<td>3636</td>
<td>0.29</td>
<td>1307</td>
<td>0.14</td>
<td>103</td>
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<tr>
<td>TRP9</td>
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<td>3488</td>
<td>0.40</td>
<td>1145</td>
<td>0.19</td>
<td>170</td>
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</tbody>
</table>

a) At 25°C.
b) Fluorescence decay law: \( K(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\tau_3) \).
Table 3. Fluorescence Anisotropies (in ps) of 17-residue peptides a),b),c)

<table>
<thead>
<tr>
<th></th>
<th>$r_1(0)$</th>
<th>$\tau_{r1}$</th>
<th>$r_2(0)$</th>
<th>$\tau_{r2}$</th>
<th>$&lt;\tau_r&gt;$ d)</th>
<th>$&lt;\tau_r&gt;$ e)</th>
<th>$&lt;\tau_r&gt;$ f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRP3</td>
<td>0.048</td>
<td>733</td>
<td>0.026</td>
<td>125</td>
<td>519</td>
<td>200</td>
<td>210</td>
</tr>
<tr>
<td>TRP6</td>
<td>0.063</td>
<td>732</td>
<td>0.025</td>
<td>102</td>
<td>553</td>
<td>333</td>
<td>440</td>
</tr>
<tr>
<td>TRP9</td>
<td>0.095</td>
<td>1076</td>
<td>0.033</td>
<td>107</td>
<td>826</td>
<td>826</td>
<td>880</td>
</tr>
</tbody>
</table>

a) At 25°C.
b) Anisotropy decay law: $r(t) = r_1(0)\exp(-t/\tau_{r1}) + r_2(0)\exp(-t/\tau_{r2})$.
c) Mean reorientation time: $<\tau_r> = [r_1(0)\tau_{r1} + r_2(0)\tau_{r2}]/[r_1(0) + r_2(0)]$.
d) From free fits.
e) From double exponential fits with $r(0)=0.12$.
f) From single exponential fits with $r(0)=0.12$.

d) The average reduced friction coefficient and rate scale are

$$
\zeta = n^{-1} \sum_{j=0}^{n-1} \frac{r_j}{l} = 0.89565, \quad \text{and} \quad \sigma = 3k_B T/6\pi \eta \zeta l^3 = 0.01310 \text{ ps}^{-1}.
$$

e) $T=300K$ in the calculations.
f) From double exponential fits with $r(0)=0.12$.

Table 4. Model calculations of rotational correlation times d),e)

<table>
<thead>
<tr>
<th></th>
<th>$&lt;\tau_r&gt;$ a)</th>
<th>$&lt;\tau_r&gt;$ b)</th>
<th>$&lt;\tau_r&gt;$ c)</th>
<th>$&lt;\tau_r&gt;$ f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRP3</td>
<td>310</td>
<td>258</td>
<td>221</td>
<td>200</td>
</tr>
<tr>
<td>TRP6</td>
<td>1077</td>
<td>815</td>
<td>625</td>
<td>333</td>
</tr>
<tr>
<td>TRP9</td>
<td>1239</td>
<td>910</td>
<td>665</td>
<td>826</td>
</tr>
</tbody>
</table>

a), b), c) Calculations made with $\zeta = 0.1$, 0.25, and 0.5, respectively.
d) The average reduced friction coefficient and rate scale are

$$
\zeta = n^{-1} \sum_{j=0}^{n-1} \frac{r_j}{l} = 0.89565, \quad \text{and} \quad \sigma = 3k_B T/6\pi \eta \zeta l^3 = 0.01310 \text{ ps}^{-1}.
$$

e) $T=300K$ in the calculations.
f) From double exponential fits with $r(0)=0.12$.

Figure 1. Normalized rotational relaxation function for a single tryptophan molecule calculated from MD system #1. A relaxation time of 5 ps is observed.
Figure 2. Normalized rotational relaxation function for a single tryptophan molecule calculated from MD system #3. Relaxation times of 40 ps for $^1L_a$ transition and 60 ps for $^1L_b$ transition are observed.

Figure 3. Normalized rotational relaxation function for a single tryptophan molecule calculated from MD system #5. Relaxation times of 40 ps for $^1L_a$ transition and 60 ps for $^1L_b$ transition are observed.
Figure 4. Dimensionless $\tau_2^l$ and $P_n/l$ values for porcine ACTH(1-39) as a function of position in the amino acid sequence. Calculated with $\zeta=0.25$ and assuming $\zeta_i=\zeta$.

Figure 5. Dimensionless $\tau_2^l$ and $P_n/l$ values for glucagon(1-29) as a function of position in the amino acid sequence. Calculated with $\zeta=0.25$ and assuming $\zeta_i=\zeta$. 
Figure 6. $\tau_2^1$ (part a) and $P_{n/l}$ (part b) for various fragments of the ACTH peptide.

Figure 7. Bond correlation times $T_2^1$ (see eqn. 3.26) of TRP9 at various values of $\zeta_r$ as a function of the virtual bond number. Calculated with $\zeta_i=\zeta$ and $\zeta=6\pi\eta|\zeta_r|$ in $\sigma$. 

Figure 8. Bond correlation times $T_{2i}$ of TRP9 at various values of $\zeta_r$ as a function of the virtual bond number. Calculated with $\zeta_i=\zeta$ and $\zeta=6\pi\eta_1(0.5)$ in $\sigma$.

Figure 9. Fluorescence anisotropy decay with its parallel and perpendicular components for TRP3. The parallel and perpendicular curves are fit simultaneously to the same parameters. The residuals for the fits are shown at the top of the figures (upper, parallel; lower, perpendicular). The $\chi_R^2$ values for the three fits are 1.2 to 1.3. The fitted parameters are listed in Table 3.
Figure 10. Relaxation lifetimes for TRP3, TRP6, and TRP9 as a function of virtual bond locations at various $\zeta_r$ calculated by taking into account the individual residue friction coefficients: a) TRP3, b) TRP6, and c) TRP9.
Figure 11. Time dependence of calculated $P_2(t)$ correlation functions for the three peptides using the individual $\zeta_i$ values as compared with the experimental data: a) TRP3, b) TRP6, and c) TRP9. The dashed lines correspond to the parameters obtained by fitting the experimental data to a sum of two exponentials with $r(0)$ fixed at 0.12 in all three cases.